Elecsys® TORCH testing
Innovative assays for high testing efficiency
With the cobas modular platform (cobas 4000 and 6000 analyzer series and cobas 8000 modular analyzer series) Roche has developed a platform concept based on a common architecture that delivers tailor-made solutions for diverse workload and testing requirements. The cobas modular platform is designed to reduce the complexity of laboratory operation and provide efficient and compatible solutions for network cooperation.

**Flexible and intelligent solutions**
- Multiple configurations with tailor-made solutions for higher efficiency and productivity
- Consolidation of clinical chemistry and immunochemistry with more than 200 parameters for cost and workflow improvements
- Future sustainability through easy adaptation to changing throughput and parameter needs
- Consistency of interaction with hardware, software and reagents for less training and more staff flexibility
- Consistency of patient results due to a universal reagent concept

### cobas 8000 modular analyzer series
Large volume
- 38 configurations

![cobas 8000 modular analyzer series](<c 302> <c 602> <c 701> <c 702>)

### cobas 6000 analyzer series
Mid volume
- 7 configurations

![cobas 6000 analyzer series](<c 501> <c 601>)

### cobas 4000 analyzer series
Low volume
- 3 configurations

![cobas 4000 analyzer series](<c 311> <c 411>)
The acronym TORCH was introduced in 1971 by Nahmias et al. to group pathogens known to cause hazardous congenital infections, namely toxoplasma, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV). This list of prenatal infections is, however, far from comprehensive, and should be supplemented with other agents. Nowadays, there are many more infections representing TORCH, such as:

- **Toxoplasmosis**
- **Other:** Treponema pallidum (syphilis), hepatitis B, hepatitis E, Coxsackie virus, Epstein–Barr virus (EBV), human parvovirus, varicella zoster and many more
- **Rubella**
- **Cytomegalovirus (CMV)**
- **Herpes simplex virus (HSV)**

Although some of these infections become chronic, they are usually asymptomatic in otherwise healthy adults. However, mothers acquiring a primary infection during pregnancy have a high risk of transmitting the pathogen to their embryo, which often has devastating consequences for the unborn. The corresponding risk in the case of secondary or reactivated infections is low.

TORCH screening is now widely requested by clinicians monitoring infants and pregnant women for congenital, perinatal and neonatal infections. The patient history, risk factors and local regulations guide the screening procedure. These tests are performed mainly in the first trimester of pregnancy, but suspect neonates may also be tested. For most TORCH pathogens the initial screening test is based on the detection of specific antibodies. The goals of TORCH testing are to determine the mother’s immune status and to help differentiate between acute and past infection in pregnancy. Pre-conception and antenatal screening play an important role in the prevention of vertically transmitted infections.

Today, many sensitive and specific tests are available for serological diagnosis of these diseases. The major challenge in serodiagnosis is the combination of high sensitivity and high specificity, although these features are mutually exclusive. To achieve a high sensitivity, the early detection of infection and the recognition of all pathogenic variants is mandatory. On the other hand, a high specificity is required to avoid uncertainty and retesting for confirmation.
Toxoplasma gondii parasite

Toxoplasma gondii is a ubiquitous intracellular parasite that can affect virtually all warm-blooded vertebrates. Infections usually lead to life-long persistence of the protozoon in muscle and nerve tissues. According to rough estimates 25 – 50 % of the global human population carry the parasite, with pronounced geographical and regional differences. Typical sources of infection are the consumption of raw or undercooked meat and the uptake of free spores released by cats into the environment.

Typical symptoms of an acute infection in otherwise healthy people are an unspecific seroconversion disease and, rarely, intensified headache, lymphadenopathy and muscle pain. Immunocompromized persons will encounter much more severe, if not fatal, sequelae. In the vast majority of cases chronic infection is asymptomatic subjectively. However, there is increasing evidence that cerebral toxoplasma cysts cause behavioral and other changes in the affected individual.

The primary hazard connected with toxoplasma infections is the vertical transmission of the pathogen by a pregnant woman with primary infection. 5 – 10 % of these pregnancies will abort spontaneously or terminate in miscarriage, another 8 – 10 % will result in the birth of an infant with severe eye and/or brain damage and 10 – 13 % of the viable babies will suffer from visual handicaps. Even if children are born without apparent symptoms, they may still develop chorioretinitis or retard mentally in childhood or young adulthood.

The incidence of primary toxoplasmosis during pregnancy in Western Europe is approximately 0.5 – 0.6 %, which, for example, is significantly higher than the incidence of congenital hypothyroidism, which affects 0.02 – 0.03 % of all viable born babies. It is for these reasons that a pre-pregnancy or prenatal screening is considered desirable, if not mandatory, in many countries and throughout large parts of the population.

Toxoplasmosis screening is based on the evidence given by serological markers, namely Toxoplasma-IgG and IgM. In contrast with many other infections, IgM antibodies can persist for years in case of chronic disease, which complicates diagnosis significantly and may require additional testing. The diagnostic strategies are very different regionally and even locally. However, an algorithm as shown below is reasonable. The testing and, if indicated, the monitoring of the pregnant woman, should start as soon as possible, as therapeutic options become available.

Even if children are born without apparent symptoms, they may still develop chorioretinitis or retard mentally in childhood or young adulthood.

The incidence of primary toxoplasmosis during pregnancy in Western Europe is approximately 0.5 – 0.6 %, which, for example, is significantly higher than the incidence of congenital hypothyroidism, which affects 0.02 – 0.03 % of all viable born babies. It is for these reasons that a pre-pregnancy or prenatal screening is considered desirable, if not mandatory, in many countries and throughout large parts of the population.

Toxoplasmosis screening is based on the evidence given by serological markers, namely Toxoplasma-IgG and IgM. In contrast with many other infections, IgM antibodies can persist for years in case of chronic disease, which complicates diagnosis significantly and may require additional testing. The diagnostic strategies are very different regionally and even locally. However, an algorithm as shown below is reasonable. The testing and, if indicated, the monitoring of the pregnant woman, should start as soon as possible, as therapeutic options become available.

Fig. 1: Suggested Toxoplasma gondii serologic diagnostic algorithm in immunocompetent individuals
The suggested screening process starts with the demonstration of specific Toxoplasma IgG and IgM. Four principal marker constellations may result:

**IgG negative, IgM negative**
This is the constellation seen in Toxoplasma-naïve individuals. These women did not have contact with Toxoplasma in the past, they do not have acquired immunity and can contract a primary infection on each contact with the pathogen. They need special medical attention and counseling. Repeated Toxoplasma testing is recommended on a regular basis. They should refrain from eating raw or undercooked meat, avoid cats and cat litter and also dogs. They should strictly adhere to common hygiene measures and should avoid eating unwashed raw fruits, vegetables and salads.

**IgG positive, IgM negative**
These women had contact with Toxoplasma in the past and are now probably carriers of non-proliferative cysts. They have acquired immunity and there is only a marginal, if any, risk of vertical transmission and subsequent embryonic damage.

**IgG negative, IgM positive**
There are two scenarios that can lead to this constellation:
1. Beginning infection, before IgG seroconversion
2. Unspecific IgM

This situation can be resolved by repeating the tests with fresh sample 3 weeks later. In the case of unspecific IgM, the result should be the same as for the initial sample. In the case of a beginning infection, IgG should be detectable by this stage.

**IgG positive, IgM positive**
This is the most difficult finding to interpret. In order to avoid being misled by persisting IgM or unspecific IgM, an IgG avidity test is recommended at this point.

If the avidity is high, an infection has taken place more than 4 months ago. The IgM result is probably due to persisting IgM or unspecific stimulation immune system. Timely screening can rule out a risk for the unborn child.

If the avidity is low to borderline, there is a risk for the unborn. An additional quantitative IgG test from a fresh sample taken 3 weeks after the first IgG test can be helpful when deciding on patient management. If the IgG titer is stable, the infection occurred more than 2 months before the first sampling. If the IgG titer is increasing significantly, an infection younger than 2 months is possibly present, which translates into a high risk for the embryo.
Rubella virus

Virology
Rubella virus is the only known Rubivirus species and belongs to the Togaviridae family. The RNA genome of Rubella virus codes for 2 non-structural and 3 structural proteins, namely the capsid protein C and the two envelope proteins E1 and E2. Its pathogenic mechanism is not yet fully understood, although there is evidence of a p53-dependent process. After replication and translation new virus particles are assembled and leave the host cell by budding.

Transmission
Postnatally the virus is spread via airborne transmission. There is no carrier state; the reservoir exists in infected people, who spread the virus by droplets from the upper respiratory tract. A patient is contagious from approximately from 1 week before to 1 week after the symptomatic phase.

Prenatally the virus can be transmitted from a pregnant woman with primary infection to the fetus through the placenta (vertical transmission).

Nosography
Rubella, also known as German measles, is the disease caused by Rubella virus. It is a typical childhood infection, usually with a mild, if not subclinical, course. Normally, children recover from it within 1 – 3 days. In adults, the disease is often associated with more pronounced manifestations, may last longer, but is self-limiting in otherwise healthy people.

By contrast, primary infection of a pregnant women during the first half of pregnancy and subsequent vertical transmission of the virus is a serious event, often leading to miscarriages or congenital rubella syndrome (CRS).

Acquired rubella
After an incubation of 2 – 3 weeks, during which the patient is contagious, the typical exanthema will develop on the face, from where it spreads over the trunk and limbs. Usually the itchy rash fades away after 1 – 3 days.

Other symptoms include low fever (rarely above 38 °C), suboccipital and posterior cervical lymphadenopathy, joint pain (often protracted in adults), headache and conjunctivitis. In about 20 % of all cases small, red papules will develop on the soft palate (Forchheimer’s sign).

Prenatal rubella virus transmission
Vertical transmission of rubella virus is a serious condition that often has disastrous consequences for the fetus, which are more severe when it occurs early in pregnancy. After passing the placenta the virus will infect the fetus, where it will stop cells from developing or will destroy them.

Many women who contract rubella within the first trimester will either have a miscarriage or a stillborn baby. Delivery is often premature with babies having a low birth weight. If an infected baby survives the birth barrier, it may present congenital rubella syndrome (CRS), which includes blindness, deafness and congenital heart failure, a patent ductus arteriosus (PDA) being the most frequent cause. Furthermore, prenatal rubella infection may entail cerebral defects, neonatal thrombocytopenia, anemia, hepatitis and skin lesions known as “blueberry muffin lesions”.

Treatment
No direct treatment is available for rubella. Therapeutic efforts focus on diminishing the discomfort associated with the disease. In newborns with a prenatal rubella infection severe manifestations are treated, if possible. For example, cataracts and congenital heart failure are often managed by surgery.

Prevention
An effective vaccine is available against rubella virus, which nowadays is usually given as part of the MMR vaccination. Every woman of childbearing age should be immune to the virus, either having had a previous infection or having been vaccinated. In countries, where rubella vaccination is common, endemics have been successfully interrupted. Occasional flare-ups result from virus influx from countries without organised vaccination programs. Additionally, in a few European countries, there is resistance against vaccination in children from some parents, including MMR vaccination.
**Diagnosis**

The serological profile after a rubella virus infection is straightforward.

Shortly after infection the virus starts to proliferate in the upper respiratory tract and is also detectable in blood. During this period patients are contagious. Approx. 2 – 3 weeks after the infection the typical exanthema and other symptoms evolve, followed by a rapid elimination of the viral load and the development of a specific IgM and IgG titer. During convalescence the IgM is usually completely eliminated, although it can also persist for several months. IgG remains elevated, normally for the rest of life, and protects against reinfections.

**IgG positive, IgM negative**

1. A significant IgG titer without any other rubella indicators is presumptive for immunity, either after a previous infection or vaccination. These women are not at risk of a prenatal infection.

2. A low, marginal titer of IgG may indicate seroconversion at a very early stage of infection, when IgM not yet detectable. If observed during pregnancy, the IgG test should be repeated a few weeks later with a fresh sample.

**IgG negative, IgM positive**

1. The patient is in the very early stages of infection, with IgM, but not IgG, having already responded. If observed during pregnancy, the test should be repeated a few weeks later with a fresh sample.

2. The IgM result may be unspecific. Again, the finding can be clarified with a test performed with a sample taken a few weeks later.

**IgG positive, IgM positive**

1. The patient is suffering from an acute infection, in particular with preceding clinical signs. The serological finding can be refined with a rubella IgG avidity test. If this constellation occurs during pregnancy the embryo is at risk. To prove a fetal infection, the virus can be confirmed by PCR in fetal blood (cordocentesis).

2. Without preceding clinical signs there is the possibility that the IgM is unspecific. To refine this suspicion a rubella IgG avidity test can be done.
Cytomegalovirus (CMV) also known as human herpes virus 5 (HHV 5) belongs to the family of herpes viruses. It is one of the world’s most widespread viruses with a regionally differing carrier prevalence between 40 and close to 100 %. CMV is transmitted parenterally through body fluids, blood transfusion or organ transplantation. Infection from person to person requires close intimate contact. CMV can be transmitted sexually, vertically before term, and perinatally. It is the most common viral cause of birth defects in industrialized countries. Like all herpes viruses CMV can persist latent within the body over long periods, with the occurrence of recidivations.

In otherwise healthy people infection usually proceeds without subjective symptoms or involves a mild seroconversion disease. A sore throat is common. A few patients will develop more noticeable symptoms such as infectious mononucleosis, glandular fever-like syndrome, prolonged fever or even mild hepatitis. Recent research is unveiling a more malicious side of CMV: Kidney cells infected with CMV seem to produce renin, which directly interferes with the RAA system, contributing to high blood pressure. Other authors suggest that CMV infection of blood vessel endothelial cells may be a major cause of atherosclerosis.

In immunocompromized patients a primary infection with CMV or a reactivation has much more aggressive consequences. A CMV-hepatitis may proceed fulminant, with the development of special forms of retinitis and colitis. The outcome is not infrequently fatal. A symptomatic CMV infection is an AIDS-defining event.

The most sinister consequences can result after a primary infection of a pregnant woman with vertical virus transmission to the unborn child. Obviously, the incidence of this hazardous event depends on the prevalence of acquired immunity in the community under consideration. For example, in the United States as many as 1 – 3 % of all pregnant women will suffer a primary CMV infection before term! And – what makes the story even worse – due to the mild clinical course of the disease, most mothers won’t even notice that they are acutely infected. 10 – 15 % of these women will give birth to an affected baby with symptoms that may include:

- low birth weight
- microcephaly
- seizures
- petechial rash
- moderate hepatosplenomegaly
- jaundice

![Fig. 3: Suggested CMV serologic diagnostic algorithm in immunocompetent individuals](image-url)
The initial lab diagnosis of CMV infection, either for screening purposes or if a concrete suspicion exists, is based on the detection of specific IgG and IgM antibodies against the virus. The age of an infection can be estimated by IgG-avidity tests. IgM alone is not always reliable for this purpose, as persisting IgM may also be present in case of a non-acute infection due to recurrences or reinfections, unspecific stimulation of immune system, etc. Confirmation is done with nucleic acid tests, in the case of prenatal screening using fetal tissue (blood or placenta).

No generally agreed screening algorithm is available for pregnancy monitoring. The algorithm presented below is certainly the best solution and covers all the aspects discussed above.

**IgG negative, IgM negative**

This is the constellation seen in CMV-naïve individuals. These women did not have contact with CMV in the past, they do not have acquired immunity and can contract a primary infection on each contact with the pathogen. They need special medical attention and counseling. Repeated CMV testing is recommended on a regular basis during pregnancy. Throughout the pregnancy, they should practice good personal hygiene, especially hand washing with soap and water, after contact with diapers or oral secretions (particularly with a child who is in day care).

**IgG negative, IgM positive**

There are two scenarios that can lead to this constellation:
1. Beginning infection, before IgG seroconversion
2. Unspecific IgM

This situation can be resolved by repeating the tests with fresh sample 3 weeks later. In the case of unspecific IgM, the result should be the same as with the initial sample. In case of a beginning infection, IgG should be detectable by this stage.

**IgG positive, IgM negative**

These women had contact with CMV in the past and have now acquired immunity. There is only a marginal, if any, risk of vertical transmission and subsequent fetal damage. However, recidivation is possible, but is very unlikely to cause fetal morbidity.

**IgG positive, IgM positive**

This is the most difficult finding to interpret. The next step is to determine IgG avidity. Depending on the corresponding gestational age this result triggers different confirmation algorithms: If the gestational age is less than, or equal to, 20 weeks and avidity is high, it is assumed that infection has occurred before conception. The initial positive IgM finding is then attributed to a persisting response or considered as unspecific. Further measures are not necessary.

If the gestational age is less than or equal to, 20 weeks and avidity is low, the possibility that infection has occurred after conception cannot be ruled out. Additional confirmatory testing is recommended.

If the gestational age exceeds 20 weeks and the IgG avidity is low to borderline, a fresh infection is possible and additional testing is recommended.

If the gestational age exceeds 20 weeks and the IgG avidity is high, then the CMV results from archived first trimester samples have to be included in the diagnostic process.

If the first trimester samples are IgM and IgG positive with high avidity, an acute infection can be excluded and further measures are not necessary.

If the first trimester samples are IgM negative and IgG positive, there is probably a non-primary infection with a low risk of vertical transmission and further measures are not necessary.

In case of all other constellations in the first trimester samples an acute infection cannot be excluded and further testing is recommended.

In order to avoid being misled by persisting IgM, an IgG avidity test is recommended at this point.

If the avidity is high, an infection has taken place more than 4 months ago. The IgM result is probably due to persisting IgM. Timely screening can rule out a risk for the unborn child.

If the avidity is low to borderline, there is a risk for the unborn child. An additional quantitative IgG test from a fresh sample taken 3 weeks after the first IgG test can be helpful when deciding on patient management: If the IgG titer is stable, the infection occurred more than 2 months before the first sampling. If the IgG titer is increasing more than twofold, an infection younger than 2 months is possibly present, which translates into a high risk for the embryo.
Herpes simplex virus (HSV)\textsuperscript{44–57}

**Virology**  
Approximately 100 Herpes viruses have been identified to date, 8 of which are pathogenic in humans.

Herpes Simplex Virus 1 (HSV 1) and Herpes Simplex Virus 2 (HSV 2) are two closely related viruses, belonging to the family of Herpesviridae, subfamily Alphaherpesviridae, genus Simplexvirus. They are human-specific. Like all herpes viruses they have a large double-stranded DNA genome that encodes more than 100 gene products. The icosahedric capsid is embedded in approx. 20 different tegument proteins and covered with an outer envelope comprising at least 10 shell proteins.

**Transmission and prevalence**  
Postnatally the transmission is usually horizontal through close contact with persons shedding virus. Not only secretions from sores can contain virus, but also saliva or genital fluids. Transmission is likely when visible symptoms are present, but can also occur from apparently asymptomatic patients.

HSV 1 is mainly transmitted via social contacts during childhood, but also sexually later in life. Already by the end of puberty high seroprevaleces of specific antibodies are observed, which increase only slightly later in life (Germany: 84 – 92 %).

HSV 2 is usually transmitted sexually by asymptomatic shedding. In health-aware individuals the seroprevalence of specific antibodies was found to be in the range of 3 – 23 % (US), while it was much higher in patients with other venereal diseases (55 %) or prostitutes (75 %).

Orofacial herpes manifestations are caused by HSV 1 in 80 % of cases and by HSV 2 in the remaining 20 %. The figures are reversed for genital herpes: HSV 1 accounts for 20 % and HSV 2 for 80 % of all cases.

Both viruses can also be transmitted vertically before birth (rare) or perinatally during delivery. Such infections may have severe, if not fatal, consequences for the fetus/newborn.

---

**Fig. 4: Incidence of congenital HSV 1 and 2 infections\textsuperscript{48, 49, 52, 54}**
Nosography
A primary infection with HSV is often associated with the development of painful watery blisters that release an infectious exudate. Typical sites are the mouth, lips (Herpes labialis) or genitals (Herpes genitalis). More severe sequelae of a primary infection are rare, but possible and include herpetic whitlow, herpes gladiatorum, ocular herpes, cerebral herpes with encephalitis, Mollaret’s meningitis, Bell’s palsy and many other disorders.

After the acute symptoms have healed, the virus is not eliminated, but the patient becomes a carrier with the infection being latent. HSV 1 and 2 are neurotropic and neuroinvasive viruses that hide from the immune system, entering the cell bodies of nerves. Recent evidence suggests that – given a certain genetic setup – a latent HSV 1 infection may even be an etiologic factor in the development of Alzheimer’s disease.

Many carriers will suffer from sporadic reactivations. In such an outbreak viruses leave the nerve cells and move via the axon to the skin, where they proliferate causing painful sores.

If transmission occurs perinatally, the newborn will most likely be infected. The undeveloped immune system is not able to fight the virus effectively, and severe consequences such as skin, eye and mouth involvement, herpes simplex encephalitis, pneumonitis, keratitis and other conditions may develop, possibly with a fatal outcome or leading to irreversible morbidity. The risk of transmission is in the range of 20 – 30 % in the case of maternal primary infection, which is often asymptomatic. For recurrences in seropositive mothers the risk is around 2 %.

Prenatal (intra-uterine) infection of the embryo is a comparably rare event, but may have very severe sequelae, including a high risk of spontaneous abortion, intra-uterine growth retardation, premature birth, fetal damage, local disseminated neurologic morbidity, intravascular coagulopathy and others. The morbidity after an HSV 2 infection is higher than after an HSV 1 infection.

In immunocompromized patients a Herpes infection easily generalizes with severe consequences for the patient.

Diagnosis
In otherwise healthy adults diagnosis is usually achieved by assessment of the clinical picture. More sophisticated diagnostic procedures, such as direct virus detection, genotyping and serological demonstration of specific antibodies, are indicated for immunocompromized patients and in the field of obstetrics.

Seroconversion in HSV 1 or 2 IgG during pregnancy involves a risk of vertical transmission. Genital herpes sores during delivery clearly suggest that a cesarean section is advisable. However, as asymptomatic virus shedding is also possible, the obstetrician has to decide whether to perform a section in case of an existing HSV 2 IgG titer.

The detection of specific IgM antibodies currently has no clinical relevance. Primary infections may occur without an immediate IgM response; on the other hand, the presence of an IgM-titer does not necessarily prove a primary infection. IgM antibodies against HSV may persist for months or years and may reappear again in the case of reactivation.

Prophylaxis
No vaccination is available at this time.

The transmission of HSV 1 through social contacts during childhood can not realistically be avoided. The sexual transmission of HSV can only be suppressed by avoiding all skin-to-skin contacts. The use of barrier methods reduces, but does not exclude transmission. As with all sexually transmitted diseases, the use of condoms and dental dams is highly recommended in non-monogamous relations. The use of antiviral medication also helps to reduce the risk of transmission.

Treatment
There is currently no curative treatment available against Herpes infections. A number of antiviral drugs (topic and atopic) can be used to ameliorate the clinical consequences of virus reactivations. Antivirals given as of gestational week 36 reduce the risk of viral shedding during delivery.
When Columbus returned from the West Indies, he had an unwanted, morbid stowaway on board: Treponema pallidum ssp. pallidum – the causative agent of syphilis. Syphilis has since spread around the world, plaguing and killing humans. The advent of antibiotics brought relief, but syphilis is now making a come-back.

**Bacteriology**

The infective agent causing syphilis is a bacterium belonging to the order of Spirochaetales, family Spirochaetaceae, genus Treponema. Spirochaetales are gram-negative spirochetes (Greek: coiled hair), which are extremely thin and can be very long. They have a tightly coiled helical structure and are motile thanks to periplasmic flagella. The genus Treponema comprises two species: pallidum and carateum, the pallidum species being subdivided into three subspecies: pallidum, endemicum and pertenue. These spirochetes are too thin to be seen under a routine microscope, although dark-field microscopy will reveal them.

T. carateum causes a disease known as pinta, T. pallidum ssp. endemicum is the infective agent in bejel and T. pallidum ssp. pertenue in yaws. Infection with T. pallidum ssp. pallidum is etiologic for syphilis. While pinta, bejel and yaws are non-venereal diseases mainly seen in undeveloped tropical and subtropical regions, syphilis is primarily a sexually, but also a vertically, transmitted disease that is prevalent worldwide. Syphilis is also known as Lues or French Disease.

T. pallidum ssp. pallidum is an intracellular pathogen, which cannot be grown in cell-free cultures in vitro. It is quite sensitive in vitro and can be killed by high temperatures (41 °C, 2 h), low temperatures (4 °C, 72 h), dryness, insecticides and changes in osmolality.

**Transmission**

Syphilis is mainly transmitted sexually, but also parenterally and perinatally, in rare cases parenterally. With respect to sexual transmission, there is a 30% chance of acquiring disease after a single exposure to an infected partner, but the transmission rate obviously also depends on the stage of the disease.
Disease stages of syphilis

In adults, syphilis proceeds in 3 stages (primary, secondary, tertiary syphilis) separated by latent phases of various duration.

The pathogen enters the host through minor lesions in the skin and then incubates for several weeks. During this period it multiplies and spreads rapidly throughout the body via lymphatic and systemic circulation. No general symptoms are involved at this stage.

The symptoms of primary syphilis appear 10-90 days (usually 3-4 weeks) after the initial contact: The host mounts an inflammatory response at the site of inoculation, resulting in the hallmark syphilitic lesion known as chancre. This ulceration is almost painless, a fact that differentiates syphilis from other ulcerating diseases such as herpes.

High levels of Treponema are found in such ulcers. Within two months the chancre will heal spontaneously, giving a false feeling of security.

2 – 10 weeks after the primary lesion, sometimes concomitantly with it, secondary syphilis will arise. During this stage, many spirochetes are spread throughout the body and a widely disseminated mucocutaneous rash will develop, which is highly contagious. A generalized immunological response with severe subjective symptoms is usually associated with this stage. Patients will suffer from pharyngeal pain, myalgia and general lymphoadenopathy, and sometimes even more severe sequelae.

Following secondary syphilis the host enters a latent period. Within a period of approx. 4 years after the infection, secondary syphilis often flares up again, albeit with milder manifestations. This so-called early latent phase merges into late latent syphilis. This stage has no clear clinical symptoms and lasts until symptoms of tertiary syphilis will develop.

Approx. 40 % of late latent patients will develop tertiary syphilis. This stage is characterized by the development of granulomatous dermal lesions (gummas), ranula, cardiovascular syphilis and neurosyphilis. Neurosyphilis develops in about 15 % of untreated cases, usually more than 5 years after the initial infection. It affects the central nervous system and the spinal chord, possibly leading to dementia, seizures and wasting. Meningitis due to Treponema may damage the brain parenchyma, resulting in progressive paralysis and impairment of the spinal cord functions. Cardiovascular involvement appears 10-40 years after initial infection with resulting myocardial insufficiency and death.

Congenital syphilis

The seroprevalence of T. pallidum during pregnancy is relatively low in Western countries (0.02 % to 4.5 %), whereas it may be much higher in other parts of the world. A dramatic increase in the incidence of congenital syphilis is currently observed in rural areas of Eastern Europe and Central Asia. There is a close correlation between the incidence of primary and secondary syphilis in women and the incidence of congenital syphilis.

Syphilis can be transmitted vertically from a seropositive mother to the fetus, usually during the 2nd and 3rd trimesters. T. pallidum will widely disseminate in the unborn leading to septicemia, often with the consequence of abortion or neonatal mortality. Children surviving the birth barrier can suffer from mental or physical problems later in life.

Early congenital syphilis, which develops within few years after birth, is associated with symptoms such as rhagades, syphilitic pemphigus, syphilitic rhinitis and osteochondritis. Late congenital syphilis can develop during early adolescence of late childhood and manifests itself in the form of e.g. inner ear hearing loss, parenchymatous keratitis, Hutchinson’s teeth (Hutchinson’s triad), gumma and central nervous system lesions.

WHO estimates that maternal syphilis causes, each year, 460,000 abortions or stillbirths, 270,000 cases of congenital syphilis, and 270,000 premature or low-birth-weight babies.

Prophylaxis and treatment

There is no vaccination available to protect from syphilis. Since syphilis is a sexually transmitted disease, practising the rules of safer sex protects against transmission. However, since T. pallidum can enter through any breaches in the skin, the use of barrier methods does not provide complete protection (e.g. syphilitic whitlow). Syphilis can be treated effectively with antibiotics, namely penicillin or tetracycline.

Diagnosis

Accurate diagnosis of syphilis is essential as it is an important health problem worldwide. The incidence is on the rise again in Western countries, particular in big cities and other hotspots. The latent phases of syphilis proceed without subjective symptoms, and each case that remains undiscovered involves the risk of serious consequences, including stillbirths, congenital syphilis, further sexual or parenteral transmission, tertiary syphilis. On the other hand, if properly diagnosed a patient can be treated effectively.

The diagnosis of symptomatic primary and secondary syphilis is often straightforward and is based on the clinical picture. Serological tests are only required for confirmation. Non-symptomatic syphilis can only be diagnosis by laboratory tests. Basically, three procedures are available:
1. Direct demonstration of T. pallidum ssp. pallidum by dark field microscopy or PCR
2. Treponemal antibody tests (HetIA, HIA, agglutination)
3. Non-treponemal tests (VDRL, RPR, HIA, agglutination)

In order to overcome the specificity shortcomings of the assays, treponemal and non-treponemal tests are often done together. A positive antibody titer indicates prior exposure to T. pallidum, while non-treponemal tests are particularly well suited for therapy monitoring.
<table>
<thead>
<tr>
<th>Glossary</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>Reduction in the ability of blood to transport oxygen. May be due to a reduction in the concentration of intact hemoglobin, hematocrit or the number of intact erythrocytes.</td>
</tr>
<tr>
<td>Avidity</td>
<td>Avidity is a measure of the stability of immune complexes, including the affinities of paratope-epitope interactions and statistical properties due to multipoint binding.</td>
</tr>
<tr>
<td>Capsid</td>
<td>A complex, usually symmetric, protein structure that coats the viral genome.</td>
</tr>
<tr>
<td>Cataract</td>
<td>Clouding of the crystalline lens or its envelope, possibly up to total opacity.</td>
</tr>
<tr>
<td>Cervical</td>
<td>Concerning the neck.</td>
</tr>
<tr>
<td>Chorioretinitis</td>
<td>Simultaneous inflammation of the conjunctiva and the choroid.</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>Pathological impairment of blood coagulation.</td>
</tr>
<tr>
<td>Colitis</td>
<td>Inflammation of the colon.</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Inflammation of the conjunctiva.</td>
</tr>
<tr>
<td>Contagious</td>
<td>Capable of transmitting disease.</td>
</tr>
<tr>
<td>Curative</td>
<td>Tending to overcome disease and promote recovery.</td>
</tr>
<tr>
<td>Dissemination</td>
<td>Spreading.</td>
</tr>
<tr>
<td>Ductus arteriosus</td>
<td>Prenatal shunt between the aorta and the pulmonary artery.</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>Inflammation of the brain.</td>
</tr>
<tr>
<td>Endemic</td>
<td>An infection in a population that is maintained without the need for external inputs.</td>
</tr>
<tr>
<td>Envelope</td>
<td>Found in some viruses: A protein layer that covers the capsid, often faking a cellular membrane.</td>
</tr>
<tr>
<td>Enteral</td>
<td>Uptake of nutrients, medication or an infectious agent via the intestine.</td>
</tr>
<tr>
<td>Exanthema</td>
<td>Acutely occurring rash.</td>
</tr>
<tr>
<td>Exudate</td>
<td>Secretion usually caused by inflammation.</td>
</tr>
<tr>
<td>Flagellum</td>
<td>A tail-like part of a bacterium employed to gain motility.</td>
</tr>
<tr>
<td>Fulminant</td>
<td>Beginning suddenly, proceeding fiercely and rapidly.</td>
</tr>
<tr>
<td>Genome</td>
<td>The total of inheritable cellular information, coded in strands of nucleic acids.</td>
</tr>
<tr>
<td>Gumma</td>
<td>Rubber-like hardened nodule, usually of the skin, that develops during tertiary syphilis.</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>Simultaneous enlargement of liver and spleen.</td>
</tr>
<tr>
<td>Horizontal transmission</td>
<td>Transmission of a pathogen between members of the same species that are not in a parent-child relationship.</td>
</tr>
<tr>
<td>Hutchinson’s triad</td>
<td>Complex of three symptoms that often occur secondarily to congenital syphilis: inner ear hearing impairment, keratitis, dental deformations.</td>
</tr>
<tr>
<td>Iatrogenic</td>
<td>Caused by medical actions.</td>
</tr>
<tr>
<td>Icosahedral symmetry</td>
<td>Most common symmetry of virus capsids. An icosahedron is a body encompassed by 20 identical triangles.</td>
</tr>
<tr>
<td>Immunocompromized</td>
<td>Patients who have a weakened, impaired or inoperative immune system. May be iatrogenic (e.g. immunosuppressive therapy) or acquired (e.g. AIDS).</td>
</tr>
<tr>
<td>Incidence</td>
<td>Number of new occurrences of a certain condition in a given population during a given time period.</td>
</tr>
<tr>
<td>Keratitis</td>
<td>Inflammation of the cornea.</td>
</tr>
<tr>
<td>Late infection</td>
<td>Equilibrium between host and pathogen. The pathogen is present but is controlled by the host’s immune system and does not cause subjective disease.</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>Pathological swelling of lymph nodes.</td>
</tr>
<tr>
<td>Meningitis</td>
<td>Inflammation of the membranes covering the brain and the spinal cord.</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>Developmental abnormality presenting as a skull with a significantly reduced volume.</td>
</tr>
<tr>
<td>MMR</td>
<td>Measles – Mumps – Rubella.</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>Disease caused by infection with Epstein-Barr Virus.</td>
</tr>
<tr>
<td>Morbidity</td>
<td>The presence of disease or illness.</td>
</tr>
<tr>
<td>Muco-cutaneous zone</td>
<td>Transition area from mucosa to skin.</td>
</tr>
<tr>
<td>Myalgia</td>
<td>Muscle pain.</td>
</tr>
<tr>
<td>Naive (pathogen)</td>
<td>Not having had any contact with a particular pathogen in the past.</td>
</tr>
<tr>
<td>Neuroinvasive</td>
<td>A virus that infects only, or preferentially, nerve cells.</td>
</tr>
<tr>
<td>Neurotropic</td>
<td>A virus that infects only, or preferentially, nerve cells.</td>
</tr>
<tr>
<td>Nosography</td>
<td>Systematic description of diseases.</td>
</tr>
<tr>
<td>Oro-facial</td>
<td>Related to the mouth and the face.</td>
</tr>
<tr>
<td>Osteochondritis</td>
<td>Painful inflammation of the bone or cartilage in a joint.</td>
</tr>
<tr>
<td>p53</td>
<td>Protein found in many malignant, but also in benignly proliferating, cells. It appears to play a role in the regulation of the cell cycle, where it controls the activity of a number of genes.</td>
</tr>
<tr>
<td>Papule</td>
<td>Circumscribed, solid elevation of skin with no visible fluid.</td>
</tr>
<tr>
<td>Parenchyma</td>
<td>The functional part of an organ.</td>
</tr>
<tr>
<td>Pemphigus</td>
<td>Blistering autoimmune disease that affects the skin and mucous membranes.</td>
</tr>
<tr>
<td>Perinatal</td>
<td>Period between the 24th week of gestation and the 7th day after birth.</td>
</tr>
<tr>
<td>Periplasm</td>
<td>See periplasmic space.</td>
</tr>
<tr>
<td>Periplasmic space</td>
<td>The space between the inner cytoplasmic membrane and the external outer membrane of certain bacteria.</td>
</tr>
<tr>
<td>Persistent IgM</td>
<td>Specific IgM persisting after convalescence from an acute infection.</td>
</tr>
<tr>
<td>Petechial rash</td>
<td>Bleeding from capillaries in the skin or mucosa leading to multiple pinhead-sized hematomas.</td>
</tr>
<tr>
<td>Pharyngeal</td>
<td>Related to the throat.</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>Inflammation of the lung.</td>
</tr>
<tr>
<td>Posterior</td>
<td>Located toward the back.</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Number of occurrences of a certain condition in a given population.</td>
</tr>
<tr>
<td>Primary infection</td>
<td>First infection of a patient with a given pathogen.</td>
</tr>
<tr>
<td>Proliferative</td>
<td>Growing, propagating.</td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin-angiotensin-aldosterone system.</td>
</tr>
<tr>
<td>Ranula</td>
<td>Cyst beneath the tongue, caused by retained saliva.</td>
</tr>
<tr>
<td>Recidivation</td>
<td>Relapse or recurrence of a disease.</td>
</tr>
<tr>
<td>Replication</td>
<td>Multiplication of genetic material, usually a genome.</td>
</tr>
<tr>
<td>Rhagades</td>
<td>Deep fissures of the skin.</td>
</tr>
<tr>
<td>Sequela</td>
<td>Complication of an acute condition.</td>
</tr>
<tr>
<td>Septicemia</td>
<td>Presence of pathogens in the bloodstream leading to sepsis.</td>
</tr>
<tr>
<td>Seroconversion</td>
<td>The point in time when a serological marker (usually an antibody) becomes detectable after an infection.</td>
</tr>
<tr>
<td>Seropositive</td>
<td>Describes a sample (or patient) that contains a detectable titer of a serological marker.</td>
</tr>
<tr>
<td>Seroprevalence</td>
<td>The prevalence of a serological marker in a given population.</td>
</tr>
<tr>
<td>Suboccipital</td>
<td>The location between the skull and the first cervical vertebra.</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Under the skin of a mucocutaneous zone.</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Platelet count below 50,000 per μL.</td>
</tr>
<tr>
<td>Translation</td>
<td>Synthesis of proteins based on information provided by genes.</td>
</tr>
<tr>
<td>Unspecific IgM</td>
<td>IgM not directed against a pathogenic epitope.</td>
</tr>
<tr>
<td>Venereal diseases</td>
<td>Sexually transmitted diseases such as syphilis and gonorrhea.</td>
</tr>
<tr>
<td>Vertical transmission</td>
<td>Transmission of a pathogen from mother to child.</td>
</tr>
</tbody>
</table>
Elecsys® TORCH testing
Innovative assays for high testing efficiency
Elecsys® TORCH testing
Innovative assays for high testing efficiency

Based on recombinant antigens and specific assay formats like µ-capture and DAGS (double antigen sandwich), Roche has been continuously developing innovative TORCH assays. These assays enable the reliable and early diagnosis of infections in prenatal screening and the recognition of opportunistic infections in immunocompromized patients.

Elecsys TORCH IgM µ-capture assays: Proven performance
• Advanced sensitivity for earlier diagnosis
• High specificity in routine and in possibly cross-reactive samples
• Good discrimination between acute and past infections due to less reactivity to persistent IgM\(^1,2\) for diagnostically more conclusive results and less unnecessary retesting
• Smart interference blocking concept for less interferences

…for early and reliable diagnosis of acute infections

Elecsys TORCH IgG DAGS assays: Unique assay design
• High specificity without compromising sensitivity: sensitivity up to 9% better than competitive assays\(^3\) resulting in less unnecessary serological follow-ups
• Clear discrimination of negative and positive results for less retesting\(^4\)

Elecsys TORCH assays: High workflow efficiency
• Integration into routine testing with efficiency, cost and workflow improvement without compromising performance
• Outstanding SWA consolidation degree with >200 parameters for clinical chemistry and immunochemistry including many innovative markers
• Broad infectious disease menu with a complete hepatitis panel (A, B and C), HIV and TORCH
• Operational efficiency: highly automated systems (e.g. autodilution), short turn around times, less retesting and confirmation testing due to high sensitivity and specificity of the TORCH assays, wide measuring ranges
• Broad range of system platforms for every lab size with consistent patient results

…for streamlined lab organization with efficiency and cost gains

Referecnes

Fig. 1: Elecsys® Toxo IgG – Excellent specificity with best-in-class sensitivity at the same time\(^{3}\)

Fig. 2: Distribution of Elecsys® CMV IgG values in daily routine samples (n=633)\(^{2}\)
**Elecsys® Toxo IgG**

**Electro-chemiluminescence immunoassay (ECLIA) for the quantitative in-vitro determination of IgG-antibodies against Toxoplasma gondii in serum and plasma**

**Indication**

Toxoplasmosis is a common infection caused by the protozoon *Toxoplasma gondii*. The infection is mainly acquired by ingestion of food or water that is contaminated with mature oocysts shed by cats or by undercooked meat containing tissue cysts. In healthy individuals, primary, acute infection is mostly a subclinical or even asymptomatic process and turns chronic, usually persisting silently for life. However, reactivation in immunocompromized people is frequently associated with severe clinical consequences. In case of primary maternal infection with *T. gondii* during pregnancy the parasite can be transmitted vertically. Possible consequences include miscarriage, still birth, malformations, inflammations, sensorial defects, retardations. If not present at birth they may develop later in life. With gestational age the probability of fetal infection increases, while the risk of severe clinical manifestations decreases. Early drug therapy in acute infection during pregnancy can prevent or ameliorate congenital damage. The diagnosis of *T. gondii* infection usually starts with the detection of anti-Toxoplasma IgG and IgM antibodies. The presence of IgG-antibodies is indicative of an acute or chronic infection. The diagnosis of acute acquired infection during pregnancy is established by a seroconversion or a significant rise in antibody titers (IgG and/or IgM) in serial samples. Often a Toxoplasma IgG avidity test is performed to collect additional information.

**Test principle: one-step double antigen sandwich (DAGS) assay (testing time 18 min)**

**Step 1** (9 minutes):

10 μL of the patient sample are incubated with a mix of biotinylated and ruthenylated monomeric SAG1. In the presence of corresponding IgG antibodies, double antigen sandwich immune complexes are formed. IgM-class antibodies do not form stable immune complexes with a monomeric antigen due to their typically low paratope affinity.

**Step 2** (9 minutes):

After the addition of streptavidin-coated paramagnetic microparticles, the DAGS complexes bind to the solid phase via biotin-streptavidin.

**Step 3** (measurement):

The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed. Luminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield depends on the properties of the antibodies in the sample.
Elecsys® Toxo IgG test characteristics

Testing time 18 min
Test principle One-step double antigen sandwich assay
Calibration 2-point
Traceability 3rd international standard ((TOXM), NIBSC, UK
Interpretation

<table>
<thead>
<tr>
<th>Status</th>
<th>IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-reactive</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>1 – 30</td>
</tr>
<tr>
<td>Reactive</td>
<td>≥ 30</td>
</tr>
</tbody>
</table>

Sample material Serum, Li-heparin, K$_3$-EDTA, Na$_2$-citrate plasma
Sample volume 10 μL
Total imprecision (NCCLS)
cobas e 411 analyzer, Elecsys® 2010 analyzer: 2.7 – 4.0 %
cobas e 601/e 602 module, E170: 3.0 – 5.7 %
Relative sensitivity

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 %</td>
<td>317</td>
</tr>
<tr>
<td>99.5 %</td>
<td>192</td>
</tr>
<tr>
<td>100 %</td>
<td>220</td>
</tr>
<tr>
<td>100 %</td>
<td>188</td>
</tr>
</tbody>
</table>
Relative specificity

<table>
<thead>
<tr>
<th>Specificity</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.8 %</td>
<td>626</td>
</tr>
<tr>
<td>98.8 %</td>
<td>242</td>
</tr>
<tr>
<td>100 %</td>
<td>159</td>
</tr>
<tr>
<td>99.0 %</td>
<td>202</td>
</tr>
</tbody>
</table>
Analytical specificity 97.8 % in a collective of 226 potentially cross-reacting samples

Suggested Toxoplasma gondii serologic diagnostic algorithm in immunocompetent individuals

Start

Perform Toxo IgG/IgM tests

IgG neg.

IgM neg.

No immunity

Avoid primary infection

Repeat testing during pregnancy

Start

IgG pos.

IgM neg.

Acquired immunity

Past infection likely

Stop

IgG neg.

IgM pos.

Beginning infection

Repeat testing ~3 weeks later

IgG pos.

IgM pos.

Unspecific IgM

Repeat IgG test ~3 weeks later

Intermediate IgG avidity

High

Toxo IgG titer

Infection > 4 months ago

Increasing (2–4 fold increase)

Recent infection < 2 months before 1st sample

Further action may be required

IgG pos.

IgM pos.

Intermediate IgG avidity

Low

Toxo IgG titer

Infection > 2 months before 1st sample

Stable

Recent infection 2–4 months ago

References


Order information

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys® Toxo IgG</td>
<td>100 tests</td>
<td>04618815</td>
</tr>
<tr>
<td>PreciControl Toxo IgG</td>
<td>8 x 1 mL</td>
<td>04618823</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>16 mL</td>
<td>11732277</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>32 mL</td>
<td>03183971</td>
</tr>
<tr>
<td>CalSet vials, empty</td>
<td>2 x 56</td>
<td>11776576</td>
</tr>
</tbody>
</table>

COBAS, COBAS E, LIFE NEEDS ANSWERS and ELECSYS are trademarks of Roche.

©2011 Roche Diagnostics Ltd.
CH-6343 Rotkreuz Switzerland
www.cobas.com
Elecsys® Toxo IgM
Electro-chemiluminescence immunoassay (ECLIA) for the qualitative in-vitro determination of IgM-antibodies against Toxoplasma gondii in serum and plasma

**Indication**
Toxoplasmosis is a common infection caused by the protozoon *Toxoplasma gondii*. The infection is mainly acquired by ingestion of food or water that is contaminated with mature oocysts shed by cats or by undercooked meat containing tissue cysts. In healthy individuals, primary, acute infection is mostly a subclinical or even asymptomatic process and turns chronic, usually persisting silently for life. However, reactivation in immunocompromized people is frequently associated with severe clinical consequences. In case of primary maternal infection with *T. gondii* during pregnancy the parasite can be transmitted vertically. Possible consequences include miscarriage, still birth, malformations, inflammations, sensorial defects, retardations. If not present at birth they may develop later in life. With gestational age the probability of fetal infection increases, while the risk of severe clinical manifestations decreases. Early drug therapy in acute infection during pregnancy can prevent or ameliorate congenital damage. The diagnosis of *T. gondii* infection usually starts with the detection of anti-Toxoplasma IgG and IgM antibodies. The presence of Toxo IgM antibodies is presumptive of an acute, recent or reactivated Toxoplasma infection. The diagnosis of acute acquired infection during pregnancy is established by a seroconversion or a significant rise in antibody titers (IgG and/or IgM) in serial samples. Often a Toxoplasma IgG avidity test is performed to collect additional information.

**Test principle: μ-capture assay (testing time 18 min)**

**Step 1 (9 minutes):**
10 μL of the patient sample are prediluted and incubated with ruthenylated recombinant polymeric SAG1. Anti-Toxo IgM forms a stable immune complex with this antigen due to multipoint binding.

**Step 2 (9 minutes):**
Streptavidin-coated paramagnetic microparticles are bound to the immune complex via biotinylated anti-human-IgM.

**Step 3 (measurement):**
The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed. Luminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield depends on the properties of the antibodies in the sample.
Elecsys® Toxo IgM test characteristics

- **Testing time:** 18 min
- **Test principle:** μ-capture assay
- **Cut-off:** Automatically calculated from 2 calibrators
- **Interpretation:**
  - Non-reactive: < 0.8 COI
  - Indeterminate: ≥ 0.8 – 1.0 COI
  - Reactive: ≥ 1.0 COI
- **Sample material:** Serum, Li-heparin, K₃-EDTA, Na-citrate plasma
- **Sample volume:** 10 μL

- **Total imprecision:**
  - *cobas e* 411 analyzer, Elecsys® 2010 analyzer: 2.5 - 5.4 %
  - *cobas e* 601/e 602 module, E170: 1.6 - 2.4 %

- **Relative sensitivity:**
  - 95.3 % (n = 170)
  - 98.8 % (m = 84)

- **Relative specificity:**
  - 98.9 % (n = 602)
  - 99.7 % (n = 295)

- **Analytical specificity:** 99.1 % in a collective of 451 potentially cross-reacting samples

Suggested *Toxoplasma gondii* serologic diagnostic algorithm in immunocompetent individuals

1. **Start**
2. Perform Toxo IgG/IgM tests
3. **IgG neg. IgM neg.**
   - No immunity
   - Avoid primary infection
   - Repeat testing during pregnancy
   - **Start**
4. **IgG pos. IgM neg.**
   - Acquired immunity
   - Past infection likely
   - **Stop**
5. **IgG neg. IgM pos.**
   - **Start**
   - Intermediate IgM avidity
   - Toxo IgG avidity
   - **Stop**
6. **IgG pos. IgM pos.**
   - Begin infection
   - **Start**
   - Unspecific IgM
   - **Stop**
7. **Repeat IgG test ~3 weeks later**
8. **Infection > 4 months ago**
   - **Stop**
   - Increasing (2-4 fold increase)
9. **Intermediate low Toxo IgG titer**
   - Stable
   - **Stop**
10. **Recent infection < 2 months before 1st sample**
    - **Stop**
    - Further action may be required
11. **Infection > 2 months before 1st sample**
    - **Stop**
    - Increasing (2-4 fold increase)
12. **Recent infection < 2 months before 1st sample**
    - **Stop**
    - Further action may be required

References

Order information
- Elecsys® Toxo IgM: 100 tests 04618858
- PreciControl Toxo IgM 1 & 2: 8 x 0.67 mL each 04618866
- Diluent Universal: 16 mL 11732277
- Diluent Universal: 32 mL 03183971
- CalSet vials, empty: 2 x 56 11776576

©2011 Roche
Roche Diagnostics Ltd.
CH-6343 Rotkreuz
Switzerland
www.cobas.com

*COBAS, COBAS E, LIFE NEEDS ANSWERS and ELECSYS are trademarks of Roche.*
Elecsys® Rubella IgG

**Electro-chemiluminescence immunoassay (ECLIA)**

**for the quantitative in-vitro determination of IgG-antibodies against rubella virus in serum and plasma**

**Indication**

Rubella virus causes German measles, a mild rash disease which commonly occurs during childhood. Postnatal infection is rarely associated with complications. However, primary infection mainly during early pregnancy is a serious condition, as vertical transmission of the virus may cause miscarriages or congenital rubella syndrome (CRS). CRS includes blindness, deafness, congenital heart disease and mental retardation. Today’s vaccination programs have considerably reduced the incidence of acute rubella and CRS. The presence of IgG antibodies to rubella virus indicates a previous exposure either by vaccination or prior rubella infection and suggests immunity. Seroconversion of specific rubella antibodies or a significant rise of the IgG titer strongly supports the diagnosis of acute rubella infection. The quantitative determination of specific IgG is used to determine the immune status to rubella.

**Test principle: one-step double antigen sandwich (DAGS) assay / γ-capture assay (testing time 18 min)**

**Step 1 (9 minutes):**

10 μL of the patient sample are incubated with monoclonal anti-human IgG antibody, rubella-like particles (RLP), a ruthenylated monoclonal anti-rubella antibody (Ab) fragment, biotinylated E1 and ruthenylated E1.

**Step 2 (9 minutes):**

After the addition of streptavidin-coated paramagnetic microparticles, the immune complexes bind to the solid phase via biotin-streptavidin.

**Step 3 (measurement):**

The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed. Luminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield depends on the rubella IgG titer in the sample.
Elecsys® Rubella IgG test characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing time</td>
<td>18 min</td>
</tr>
<tr>
<td>Test principle</td>
<td>One-step double antigen sandwich assay (DAGS) γ-capture assay</td>
</tr>
<tr>
<td>Calibration</td>
<td>2-point</td>
</tr>
<tr>
<td>Traceability</td>
<td>1st international standard, human anti-rubella Ig RUBI-1-94 (NIBSC)</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Non-reactive: &lt;10 IU/mL, Reactive: ≥10 IU/mL</td>
</tr>
<tr>
<td>Sample material</td>
<td>Serum, Li-heparin, K3-EDTA, Na-citrate plasma</td>
</tr>
<tr>
<td>Sample volume</td>
<td>10 μL</td>
</tr>
<tr>
<td>Total imprecision</td>
<td>cobas e 411 analyzer, Elecsys® 2010 analyzer: 3.4 – 6.4 %</td>
</tr>
<tr>
<td></td>
<td>cobas e 601/e 602 module, E170: 3.2 – 4.3 %</td>
</tr>
<tr>
<td>Relative sensitivity</td>
<td>100 % (n = 514) 99.9 % (n = 978) 100 % (n = 120) 100 % (n = 20)</td>
</tr>
<tr>
<td>Relative specificity</td>
<td>97.4 % (n = 38) 100 % (n = 18) 100 % (n = 78) 100 % (n = 769)</td>
</tr>
</tbody>
</table>

Serological profile after rubella infection

![Diagram showing virus and antibody levels over time]

Order information

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys® Rubella IgG</td>
<td>100 tests</td>
<td>04618793</td>
</tr>
<tr>
<td>PreciControl Rubella IgG 1 &amp; 2</td>
<td>8 x 1 mL each</td>
<td>04618807</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>16 mL</td>
<td>11732277</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>32 mL</td>
<td>03183971</td>
</tr>
<tr>
<td>CalSet vials, empty</td>
<td>2 x 56</td>
<td>11776576</td>
</tr>
</tbody>
</table>

COBAS, COBAS E, LIFE NEEDS ANSWERS and ELECSYS are trademarks of Roche.

©2011 Roche

Roche Diagnostics Ltd.
CH-6343 Rotkreuz
Switzerland
www.cobas.com
**Elecsys® Rubella IgM**

*Electro-chemiluminescence immunoassay (ECLIA) for the qualitative in-vitro determination of IgM-antibodies against rubella virus in serum and plasma*

**Indication**
Rubella virus causes German measles, a mild rash disease which commonly occurs during childhood. Postnatal infection is rarely associated with complications. However, primary infection mainly during early pregnancy is a serious condition, as vertical transmission of the virus may cause miscarriages or congenital rubella syndrome (CRS). CRS includes blindness, deafness, congenital heart disease and mental retardation. Today’s vaccination programs have considerably reduced the incidence of acute rubella and CRS. The presence of IgM antibodies to rubella virus usually indicates an acute infection, but may also be unspecific or persistent. Seroconversion of specific rubella antibodies strongly supports the diagnosis of acute rubella infection.

---

**Test principle: μ-capture assay (testing time 18 min)**

**Step 1 (9 minutes):**
10 μL of the patient sample are prediluted and incubated with biotinylated anti-human IgM and rubella-like particles (RLP). Anti-rubella IgM forms stable immune complexes with RLPs due to multipoint binding.

**Step 2 (9 minutes):**
Ruthenylated rubella-specific antibodies are added together with streptavidine-coated paramagnetic microparticles. The antibodies bind to free sites on the RLP, while the microparticles are bound to the complex via the biotin on the capture antibody.

**Step 3 (measurement):**
The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed. Luminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield depends on the properties of the antibodies in the sample.
Elecsys® Rubella IgM test characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing time</td>
<td>18 min</td>
</tr>
<tr>
<td>Test principle</td>
<td>μ-capture assay</td>
</tr>
<tr>
<td>Cut-off</td>
<td>Automatically calculated from 2 calibrators</td>
</tr>
<tr>
<td>Interpretation</td>
<td></td>
</tr>
<tr>
<td>Non-reactive:</td>
<td>&lt;0.8 COI</td>
</tr>
<tr>
<td>Indeterminate:</td>
<td>≥0.9 – 1.0 COI</td>
</tr>
<tr>
<td>Reactive:</td>
<td>≥1.0 COI</td>
</tr>
<tr>
<td>Sample material</td>
<td></td>
</tr>
<tr>
<td>Serum, Li-heparin, K⁺-EDTA, plasma</td>
<td></td>
</tr>
<tr>
<td>Sample volume</td>
<td>10 μL</td>
</tr>
<tr>
<td>Total imprecision</td>
<td></td>
</tr>
<tr>
<td>cobas e 411 analyzer, Elecsys® 2010 analyzer</td>
<td>1.9 – 4.1 %</td>
</tr>
<tr>
<td>cobas e 601/e 602 module, E170:</td>
<td>2.7 – 10.9 %</td>
</tr>
<tr>
<td>Sensitivity in early acute infection (&lt; 30 days)</td>
<td></td>
</tr>
<tr>
<td>Non-reactive:</td>
<td>80 % (n = 84)</td>
</tr>
<tr>
<td>Relative specificity</td>
<td>96 % (n = 25)</td>
</tr>
<tr>
<td>Reactive:</td>
<td>98.7 % (n = 554)</td>
</tr>
<tr>
<td>Analytical specificity</td>
<td>99.0 % (n = 993)</td>
</tr>
<tr>
<td>Relative specificity</td>
<td></td>
</tr>
<tr>
<td>Analytical specificity</td>
<td>98.99 % in 390 potentially cross-reacting samples</td>
</tr>
</tbody>
</table>

Serological profile after rubella infection

![Serological profile graph](image)

Order information

<table>
<thead>
<tr>
<th>Item</th>
<th>Qty</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys® Rubella IgM</td>
<td>100 tests</td>
<td>04618831</td>
</tr>
<tr>
<td>PreciControl Rubella IgM 1 &amp; 2</td>
<td>4 x 1 mL each</td>
<td>04618840</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>16 mL</td>
<td>11732277</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>32 mL</td>
<td>03183971</td>
</tr>
<tr>
<td>CalSet vials, empty</td>
<td>2 x 56</td>
<td>11776576</td>
</tr>
</tbody>
</table>

COBAS, COBAS E, LIFE NEEDS ANSWERS and ELECSYS are trademarks of Roche.

©2011 Roche

Roche Diagnostics Ltd.
CH-6343 Rotkreuz
Switzerland
www.cobas.com
Elecsys® CMV IgG

Electro-chemiluminescence immunoassay (ECLIA) for the quantitative in-vitro determination of IgG-antibodies against CMV in serum and plasma

Indication

Cytomegalovirus (CMV) is a herpes virus that is ubiquitous in all human populations. Transmission occurs mainly through incorporation of virus-loaded body fluids. The global prevalence of seropositive adults ranges from 40 – 100 %. In healthy individuals, primary, acute infection is mostly a subclinical or even asymptomatic process and turns latent. Reactivation in immunocompromized people is frequently associated with severe clinical consequences. In case of primary maternal infection with CMV during pregnancy the virus can be transmitted vertically. Consequences include severe fetal damage, growth and mental retardations, jaundice and CNS abnormalities. If unsuspicous at birth, hearing defects or cognitive deficits may develop later in life. There is currently no generally accepted therapy available. The diagnosis of CMV infection usually starts with the detection of anti-CMV IgG and IgM antibodies. The detection of CMV IgG antibodies is an indicator of a past infection. The time of infection can roughly be estimated by a CMV IgG avidity test. Seroconversion in CMV IgG shows a recent infection.

Test principle: one-step double antigen sandwich (DAGS) assay (testing time 18 min)

Sample

<table>
<thead>
<tr>
<th>Anti-CMV IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotinylated recombinant pp150, pp28, p52, p38</td>
</tr>
</tbody>
</table>

Streptavidin-coated microparticle

| Ruthenylated recombinant pp150, pp28, p52, p38 |

Step 1 (9 minutes):

20 μL of the patient sample are incubated with a mix of biotinylated and ruthenylated monomeric CMV antigens. In the presence of corresponding IgG antibodies, double antigen sandwich immune complexes are formed. Following a statistical distribution these sandwiches can carry biotin and the ruthenium label simultaneously. IgM-class antibodies do not form stable immune complexes with a monomeric antigen due to their typically low paratope affinity.

Step 2 (9 minutes):

After the addition of streptavidin-coated paramagnetic microparticles, the DAGS complexes bind to the solid phase via biotin-streptavidin.

Step 3 (measurement):

The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed. Luminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield depends on the properties of the antibodies in the sample.
Elecsys® CMV IgG test characteristics

Testing time 18 min
Test principle One-step double antigen sandwich assay (DAGS)
Calibration 2-point
Interpretation
Non-reactive: < 0.5 U/mL
Indeterminate: 0.5 – 1.0 U/mL
Reactive: ≥ 1 U/mL
Sample material Serum, Li-heparin, K$_2$-EDTA, K$_3$-EDTA, plasma
Sample volume 20 μL
Total imprecision
[cobas e 411 analyzer, Elecsys® 2010 analyzer: 3.2 – 3.9 %]
[cobas e 601/e 602 module, E170: 3.2 – 4.5 %]
Agreement with a commercially available method
98.9 % (n = 532)
98.8 % (n = 616)
99.4 % (n = 520)
Analytical specificity 96.6 % in a collective of 437 potentially cross-reacting samples

Suggested CMV serologic diagnostic algorithm in immunocompetent individuals

References

Order information
Elecsys® CMV IgG 100 tests 04784596
PreciControl CMV IgG 1 & 2 8 x 1 mL each 04784600
Diluent Universal 16 mL 11732277
Diluent Universal 32 mL 03183971
CalSet vials, empty 2 x 56 11776576
**Elecsys® CMV IgM**

**Electro-chemiluminescence immunoassay (ECLIA) for the qualitative in-vitro determination of IgM-antibodies against CMV in serum and plasma**

**Indication**
Cytomegalovirus (CMV) is a herpes virus that is ubiquitous in all human populations. Transmission occurs mainly through incorporation of virus-loaded body fluids. The global prevalence of seropositive adults ranges from 40 – 100 %. In healthy individuals primary, acute infection is mostly a subclinical or even asymptomatic process and turns latent. Reactivation in immunocompromized people is frequently associated with severe clinical consequences. In case of primary maternal infection with CMV during pregnancy the virus can be transmitted vertically. Consequences include severe fetal damage, growth and mental retardations, jaundice and CNS abnormalities. If unsuspicious at birth, hearing defects or cognitive deficits may develop later in life. There is currently no generally accepted therapy available. The diagnosis of CMV infection usually starts with the detection of anti-CMV IgG and IgM antibodies. Samples being reactive for IgM antibodies indicate an acute, recent or reactivated infection. A positive IgM result in combination with a low avidity index for IgG is an indication of a primary CMV infection within the last 4 months. Seroconversion to CMV IgM also establishes the diagnosis of a recent CMV infection.

**Test principle: μ-capture assay (testing time 18 min)**

**Step 1** (9 minutes): 10 µL of the patient sample are prediluted and incubated with biotinylated anti-human-IgM.

**Step 2** (9 minutes): Ruthenylated multimeric pp150, p52 is added together with streptavidine-coated paramagnetic microparticles. Anti-CMV IgM forms stable immune complexes with these antigens due to multipoint binding which are bound to the solid phase via biotin-strapavidin.

**Step 3** (measurement): The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed. Luminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield depends on the properties of the antibodies in the sample.
Elecsys® CMV IgG test characteristics

- **Testing time**: 18 min
- **Test principle**: μ-capture assay
- **Cut-off**: Automatically calculated from 2 calibrators
- **Interpretation**
  - Non-reactive: <0.7 COI
  - Indeterminate: ≥0.7 – 1.0 COI
  - Reactive: ≥1.0 COI
- **Sample material**: Serum, Li-heparin, K3-EDTA, K2-EDTA, plasma
- **Sample volume**: 10 μL
- **Total imprecision**
  - cobas e 411 analyzer, Elecsys® 2010 analyzer: 2.4 – 5.3 %
  - cobas e 601/e 602 module, E170: 3.8 – 6.1 %
- **Sensitivity**
  - 93.0 % (n = 114)
  - 96.5 % (n = 57)
  - 91.2 % (n = 34)
  - 93.1 % (n = 29)
  - 92.3 % (n = 52)
- **Specificity in routine samples**
  - 98.8 % (n = 501)
  - 97.1 % (n = 591)
  - 97.0 % (n = 507)
- **Analytical specificity**
  - 92.3 % in 413 potentially cross-reacting samples

Suggested CMV serologic diagnostic algorithm in immunocompetent individuals

Start

Perform CMV IgG/IgM tests

IgG pos. / IgM pos.

IgG avidity ≤ 20 w

Gestational age > 20 w

IgG avidity low to borderline

High risk of transmission

Possibly additional testing

Maternal viremia
  - Virus isolation
  - Blood PCR

Fetal well-being
  - Ultrasound
  - MRI

Invasive testing
  - Amniocentesis
  - Cordocentesis

IgG neg. / IgM pos.

Low avidity

IgG neg. / IgM pos.

High avidity

IgG pos. / IgM pos.

Non-primary infection low risk of transmission

Stop

References


Order information

<table>
<thead>
<tr>
<th>Test</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys® CMV IgM</td>
<td>100 tests</td>
</tr>
<tr>
<td>PreciControl CMV IgM 1 &amp; 2</td>
<td>8 x 1 mL each</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>16 mL</td>
</tr>
<tr>
<td>Diluent Universal</td>
<td>32 mL</td>
</tr>
<tr>
<td>CalSet vials, empty</td>
<td>2 x 56</td>
</tr>
</tbody>
</table>

COBAS, COBAS E, LIFE NEEDS ANSWERS and ELECSYS are trademarks of Roche.
CMV IgG Avidity

Electro-chemiluminescence immunoassay (ECLIA) for the determination of the avidity of IgG antibodies to CMV in human serum and plasma

**Indication**
Sample being reactive for anti-CMV IgG and IgM may indicate an acute, recent or reactivated infection. Since symptomatic congenital infection in the fetus is mostly due to intrauterine transmission following primary maternal infection, differential diagnosis of primary versus recurrent infection, unspecific IgM or persistence of CMV-specific IgM antibody is crucial for the management of such a pregnancy. Antibodies produced at an early stage during primary response have lower antigen avidity than those produced at a later stage. Since low avidity is encountered up to approx. 18–20 weeks after the onset of symptoms in immunocompetent patients and is subject to interindividual variation, avidity testing should be performed at an early stage of gestation. A low-avidity anti-CMV IgG detected before the 16th–18th week of pregnancy, together with a positive anti-CMV IgM, is strong evidence of a recent primary infection, whereas a high avidity index would be considered a good indicator of past infection. A high avidity result later in gestation cannot rule out a primary infection at an earlier stage of the pregnancy.

**Reference - Test principle:** one-step double antigen sandwich assay (DAGS, assay time 18 min)

**Avidity - Test principle:** one-step double antigen sandwich assay under chaotropic conditions (DAGS, assay time 18 min)

**Step 1** (9 minutes):
20 μL of the patient sample are incubated with a mix of biotinylated and ruthenylated monomeric CMV antigens. Double antigen sandwich immune complexes are formed in the presence of corresponding IgG antibodies.

**Step 2** (9 minutes):
After the addition of streptavidin-coated paramagnetic microparticles, the DAGS complexes bind to the solid phase via biotin-streptavidin.

**Step 3** (measurement):
The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action.

50 μL of the patient sample are mixed with 50 μL of DilCMVAv. 20 μL of the diluted patient sample are incubated with a mix of biotinylated and ruthenylated monomeric CMV antigens. Double antigen sandwich immune complexes are formed in the presence of high-avidity IgG antibodies. IgG of low avidity cannot form stable immune-complexes under the prevailing conditions.
Result calculation
The analyzer automatically calculates the analyte concentration of each sample in U/mL for both measurements (reference measurement and DilCMVAv treated measurement). Calculation of avidity [Avi %]:

\[
\text{Avidity [Avi %]} = \frac{\text{result DilCMVAv treated measurement [U/mL]}}{\text{result reference measurement [U/mL]}} \times 100\%
\]

CMV IgG Avidity test characteristics

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay time</td>
<td>2 x 18 min (in parallel)</td>
</tr>
<tr>
<td>Test principle</td>
<td>One-step double antigen sandwich assay (DAGS) under various buffer conditions</td>
</tr>
<tr>
<td>Interpretation</td>
<td>2-point</td>
</tr>
<tr>
<td>Low avidity</td>
<td>&lt; 45.0 Avi %</td>
</tr>
<tr>
<td>Grey zone</td>
<td>45.0 – 54.9 Avi %</td>
</tr>
<tr>
<td>High avidity</td>
<td>≥ 55.0 Avi %</td>
</tr>
<tr>
<td>Sample material</td>
<td>Serum, Li-heparin, K\text{-}, K\text{\text{-}}-EDTA plasma</td>
</tr>
<tr>
<td>Sample volume</td>
<td>1 x 20 μL and 1 x 50 μL</td>
</tr>
<tr>
<td>Clinical sensitivity [95% confidence limit]</td>
<td></td>
</tr>
<tr>
<td>Clinical specificity [95% confidence limit]</td>
<td></td>
</tr>
</tbody>
</table>

Suggested CMV serologic diagnostic algorithm in immunocompetent individuals

COBAS, COBAS E and LIFE NEEDS ANSWERS are trademarks of Roche.

©2011 Roche

Roche Diagnostics Ltd.
CH-6343 Rotkreuz
Switzerland
www.cobas.com

References
Elecsys® HSV-1 IgG

Electro-chemiluminescence immunoassay (ECLIA) for the in-vitro determination of IgG antibodies to Herpes Simplex Virus type 1 (HSV-1)

**Indication**

Herpes simplex virus 1 (HSV-1) is mainly transmitted via social contacts during childhood, but also sexually later in life. The prevalence of HSV-1 infections in the general population is estimated to be around 70-90%. A primary infection with HSV is often associated with the development of painful watery blisters that release an infectious exudate. Typical sites are the mouth, lips (herpes labials) or genitals (herpes genitalis). Recurrent skin lesions are the hallmark of HSV pathogenesis. Orofacial herpes manifestations are usually caused by HSV-1, whereas genital herpes is mainly caused by HSV-2. HSV-1 and HSV-2 can also be transmitted vertically before birth or perinatally during delivery. Such infections may have severe, if not fatal, consequences for the fetus/newborn. Subclinical viral shedding and unrecognized infections seem to be major factors in transmission.

**Test principle: Double antigen sandwich assay (DAGS, testing time 18 min)**

- **Step 1 (9 minutes):**
  20 μL of the patient sample are incubated with a mix of biotinylated and ruthenylated HSV-1 antigens. Double antigen sandwich immune complexes are formed in the presence of corresponding antibodies.

- **Step 2 (9 minutes):**
  After the addition of streptavidin-coated paramagnetic microparticles, the DAGS complexes bind to the solid phase via biotin-streptavidin.

- **Step 3 (measurement):**
  The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed by ProCell. Luminescence is then induced by applying a voltage and measured with a photomultiplier.
Elecsys® HSV-1 IgG characteristics

Analyzer compatibility

- cobas e 601 module
- cobas e 602 module
- cobas e 411 analyzer
- MODULAR ANALYTICS E170
- Elecsys® 2010 analyzer

Assay time 18 min

Test principle DAGS assay

Cut-off automatically calculated from 2 calibrators

Sample material Serum, Li-heparin plasma, K₂-EDTA plasma, K₃-EDTA plasma

Sample volume 20 μL

Total imprecision

- cobas e 411 analyzer, Elecsys® 2010 analyzer: 2.5 – 2.7 %
- cobas e 601/e 602 module, E170: 1.6 – 2.2 %

Expected values

- Non-reactive: <0.6 COI
- Gray-zone: ≥0.6 - <1.0 COI
- Reactive: ≥1.0 COI

For quality control, use Elecsys® PreciControl HSV. The controls 1 and 2 should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

On-board stability

28 days

Elecsys® HSV-1 IgG key performance data

A. Pregnancy screening (n = 400)

Relative specificity

- Elecsys 100.0 %
- Comparator 92.4 %

Relative sensitivity

- Elecsys 95.6 %
- Comparator 100.0 %

B. Sexually active adults (n = 300)

Relative specificity

- Elecsys 100.0 %
- Comparator 92.6 %

Relative sensitivity

- Elecsys 100.0 %
- Comparator 100.0 %

Frozen samples analyzed by commercially available HSV-1 IgG assays were tested with the HSV-1 IgG Elecsys®. Resolution of discordant samples was done using a commercially available immunoblot assay. Gray-zone samples as well as inconclusive samples (i.e. concordant results with Elecsys® HSV-1 IgG and comparison method but discordant immunoblot results) were excluded from calculation.

Order information

- Elecsys® HSV-1 IgG 100 tests 05572185 190
- Elecsys® PreciControl HSV For 4 x 3 mL 05572207 190
- Control Set Vials, empty 2 x 56 03142949 122

COBAS, COBAS E, LIFE NEEDS ANSWERS, ELECSYS and MODULAR are trademarks of Roche.

©2011 Roche

Roche Diagnostics Ltd.
CH-6343 Rotkreuz
Switzerland
www.cobas.com
Elecsys® HSV-2 IgG

Electro-chemiluminescence immunoassay (ECLIA) for the in-vitro determination of IgG antibodies to Herpes Simplex Virus type 2 (HSV-2)

Indication
Herpes simplex viruses 2 (HSV-2) are usually transmitted sexually by asymptomatic shedding and account for approximately 80% of all cases of genital herpes. The prevalence of HSV-2 infections in the general population is estimated to be around 17-25%, but can be higher in specific risk groups like AIDS-patients and female sex workers. HSV-2 infection is a risk factor for HIV transmission and is associated with an increased risk of acquisition of HIV. Neonatal herpes has the most severe implications and is usually acquired during the intrapartum period through exposure in the genital tract. Subclinical viral shedding and unrecognized infections seem to be major factors in transmission. Genital HSV infection is frequently not recognized and diagnosis based on the clinical presentation alone has a low sensitivity. Type-specific serologic tests allow the identification of silent carriers of HSV-2 infection in patients with or without pre-existing antibodies to HSV-1.

Test principle: Double antigen sandwich assay (DAGS, testing time 18 min)

Sample anti-HSV-2

Streptavidin-coated microparticle

Step 1 (9 minutes):
20 μL of the patient sample are incubated with a mix of biotinylated and ruthenylated HSV-2 antigens. Double antigen sandwich immune complexes are formed in the presence of corresponding antibodies.

Step 2 (9 minutes):
After the addition of streptavidin-coated paramagnetic microparticles, the DAGS complexes bind to the solid phase via biotin-streptavidin.

Step 3 (measurement):
The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed by ProCell. Luminescence is then induced by applying a voltage and measured with a photomultiplier.
Elecsys® HSV-2 IgG characteristics

Analyzer compatibility

Assay time
18 min

Test principle
DAGS assay

Cut-off
automatically calculated from 2 calibrators

Sample material
Serum, Li-heparin plasma, K<sub>2</sub>-EDTA plasma, K<sub>3</sub>-EDTA plasma

Sample volume
20 µL

Total imprecision

Expected values

Quality control procedure
For quality control, use Elecsys® PreciControl HSV. The controls 1 and 2 should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration.

On-board stability
28 days

Elecsys® HSV-2 IgG key performance data

A. Pregnancy screening (n = 400)

Relative specificity

Relative sensitivity

B. Sexually active adults (n = 300)

Relative specificity

Relative sensitivity

Frozen samples analyzed by commercially available HSV-2 IgG assays were tested with the HSV-2 IgG Elecsys®. Resolution of discordant samples was done using a commercially available immunoblot assay. Gray-zone samples as well as inconclusive samples (i.e. concordant results with Elecsys® HSV-2 IgG and comparison method but discordant immunoblot results) were excluded from calculation.

Order information

Elecsys® HSV-2 IgG 100 tests 05572193 190
Elecsys® PreciControl HSV For 4 x 3 mL 05572207 190
Control Set Vials, empty 2 x 56 03142949 122

COBAS, COBAS E, LIFE NEEDS ANSWERS, ELECSYS and MODULAR are trademarks of Roche.

©2011 Roche
Roche Diagnostics Ltd.
CH-6343 Rotkreuz
Switzerland
www.cobas.com
Elecsys® HSV-1 IgG and HSV-2 IgG immunoassays
Type-specific assays for reliable diagnosis and assessment of the immune status
HSV-1 is mainly transmitted via social contacts during childhood, but also sexually later in life. Its prevalence in the general population is around 70-90%. HSV-2 is usually transmitted sexually and has a prevalence of around 17-25% in the general population. However, prevalence can be much higher in specific risk groups like AIDS-patients and female sex workers. Orofacial herpes manifestations are usually caused by HSV-1, whereas genital herpes is mainly caused by HSV-2. HSV-1 and HSV-2 can also be transmitted vertically before birth or perinatally during delivery. Such infections may have severe, if not fatal, consequences for the fetus/newborn. Subclinical viral shedding and unrecognized HSV-infections seem to be major factors in transmission.

Type-specific assays for reliable differentiation between HSV-1 and HSV-2

Two Elecsys® HSV IgG assays available:

- One for the detection of HSV-1 IgG and one for the detection of HSV-2 IgG
- Only type-specific serologic tests allow the identification of silent carriers infected with one subtype with or without pre-existing antibodies to the other subtype

Advantages:

- Asymptomatic HSV-2 patients can be educated to recognize symptomatic disease
- HSV-2 transmission can be reduced by education of patients
- Allows treatment that is specific for either HSV-1 or HSV-2 infection

High specificity for less retesting in clinical routine and pregnancy screening

A. Pregnancy screening (n = 400)

<table>
<thead>
<tr>
<th>HSV</th>
<th>Elecsys</th>
<th>Comparator</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV 1</td>
<td>99.6%</td>
<td>99.4%</td>
</tr>
<tr>
<td>HSV 2</td>
<td>95.6%</td>
<td>92.6%</td>
</tr>
</tbody>
</table>

B. Sexually active adults (n = 300)

<table>
<thead>
<tr>
<th>HSV</th>
<th>Elecsys</th>
<th>Comparator</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV 1</td>
<td>99.7%</td>
<td>99.2%</td>
</tr>
<tr>
<td>HSV 2</td>
<td>99.6%</td>
<td>99.2%</td>
</tr>
</tbody>
</table>

The word “relative” refers to comparing the results of the Elecsys® HSV-1 IgG and HSV-2 IgG assays with those of comparator assays. Resolution of discordant samples was done using a commercially available immunoblot assay. Gray-zone samples as well as inconclusive samples were excluded from calculation.

Completing Roche’s TORCH portfolio

The comprehensive assay menu for consolidated pregnancy testing

Elecsys® 2010 analyzer, MODULAR ANALYTICS E170, cobas e 411 and cobas e 601 analyzers

<table>
<thead>
<tr>
<th>Test</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxo IgG</td>
<td>Toxo Avidity*</td>
<td>CMV IgM</td>
</tr>
<tr>
<td>Toxo IgM</td>
<td>CMV IgG</td>
<td></td>
</tr>
<tr>
<td>HSV-1 IgG</td>
<td>HSV-2 IgG</td>
<td></td>
</tr>
</tbody>
</table>

*under development

COBAS, COBAS E, LIFE NEEDS ANSWERS, ELECSYS and MODULAR are trademarks of Roche.

©2011 Roche

Roche Diagnostics Ltd.
CH-6343 Rotkreuz
Switzerland
www.cobas.com